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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 CFR 1.53 (b)(2).

Docket Number	26360	Type a plus sign (+) inside this box -> +

INVENTOR(s) / APPLICANT(s)

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TITLE OF THE INVENTION (280 characters max)

PLANT TRICHOMES EXPRESSING FOREIGN PROTEINS AND USES THEREOF

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification	*Number of Pages*	16	☑ Applicant is entitled to Small Entity Status	
☐ Drawing(s)	*Number of Sheets*	--	☑ Other (specify)	5 CLAIMS

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☐ A check or money order is enclosed to cover the filing fees		FILING FEE AMOUNT ($)	$ 80.-
☑ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number:	50-1407		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No

☐ Yes, the name of the US Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date June 17, 2003

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REGISTRATION NO. (if appropriate) 25,457

☐ Additional inventors are being named on separately numbered sheets attached hereto

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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APPLICATION FOR PATENT

Inventors: Gil Ronen, Larisa Rabinovich, Rafi Meissner, Hagai Karchi.

Title: Plant trichomes expressing foreign proteins and uses thereof.

FIELD AND BACKGROUND OF THE INVENTION

Trichomes are hairy like epidermal multi-cell structures found on the outer surface of leaves, stems and flowers of about 20-30% of plant species. The trichomes serve mainly to physically prevent insects from attacking the tissues and for providing defense against several pests by accumulating or exuding natural pesticides, mainly alkaloids and terpenes (1,2). The massive production of metabolites is unique to the trichome cells (3). Another type of trichomes exists in several species, such as tomato and potato. These trichomes accumulate unique compounds in associated glands (round cell structures) on the top of each trichome. When an insect is landing on a leaf surface and touches those trichomes they discharged and their inner compounds are released and damaging the insect (reviewed in 4). For the mass production, accumulation and secretion of the compounds, specific machinery was evolved, including DNA sequences representing genes (5,6) and promoters (7,8,9) preferably acting in trichome cells, as well as cells organelles suited for accumulation and secretion of mass products. For example, trichome exudates can reach 16% of total dry weight of leaves of a certain tobacco species (10). In another case, a single protein can reach 60% of total proteins or a concentration of 14 mg/mL in the trichome content of a solanum species (11,12). These genetic tools and cell machinery can be exploited for the tissue specific production and accumulation of natural and heterologous proteins as well as chemicals (6). One major use can be the heterologous expression and harvesting of human or mammalian proteins for the use as therapeutics, in what is known as Molecular Farming or Bio-Farming. The desired compounds could be mechanically harvested from the surface of the leaves that may be re-synthesized in the same or in newly grown trichomes. Alternatively, the new

compounds produced can be beneficial for the plant itself, for example by increasing resistance against pests such as insects, bacteria and fungi (6). Last, structurally modified trichomes serve as the source tissue for fibers of cotton. It has been shown that the promoter sequences of cotton fiber specific genes are directing GUS expression to the trichome cells of tobbaco plants (7,9). Alteration of trichomes structure or chemistry, for example by increasing cotton trichome length or by producing pigments could be beneficial for the cotton fiber industry.

Natural chemicals of trichomes are already used as flavor, aroma, medicinals, pesticides and cosmetic ingredients (13,14). Natural chemicals content was altered using antisense and co-supression methods (6). However, enzymatic modifications of trichomes compounds, by expressing genes in trichome cells via genetic engineering, to produce other useful compounds, had never been shown before.

Several limitations had narrowed so far the use of plant trichomes for commercially production of heterologous proteins and novel chemicals:

1. The production yield of proteins. Trichomes protein yield is very limited and to our knowledge there is no existing method that enables the significant increase of the production and accumulation of proteins in trichomes. Although there are already known promoter sequences that direct protein synthesis to trichomes (7,8,9), yet it was never shown that the produced proteins were accumulating to higher levels compared to normal levels of accumulation of a single protein.

2. Existing harmful compounds. Trichomes usually produce mix of several metabolites, some of them can damage the harvesting and purification of desired compounds produced in trichomes (See material and methods in 12). Reducing harmful products, such as phenol and alkaloids, must be achieved to improve harvesting and collection of other desired products.

3. Environmental risks. The production of novel compounds in plants is always involving risks of escape of genetic material (pollen and seeds) to the environment, with potential damage to other organisms (plants, insects animals, human). Hence, when producing novel compounds one should consider the elimination of the possible spread of the new genetic material.

SUMMARY OF THE INVENTION

This invention includes several methods for massive increase in the synthesis, accumulation and harvesting of chemicals and proteins in plant trichomes, while reducing the chances of spreading the new established genetic material. Mass production is an absolute need for commercial value system that produces biology material. To overcome today limitation in production, accumulation and harvesting of biology material produced in trichomehe cells, the following methods are developed. The methods enable:

1. The massive expression of genes in trichome cells, by identifying novel, strong promoters and other regulatory elements.

2. Directing the translated polypeptides into unique cell compartment in the trichome, such as leucoplasts, for increasing the tissue capacity of the desired compounds. This will be done by identifying novel polypeptides which act as signal peptides that direct the produced protein into chloroplasts and leucoplasts.

3. Elevating the harvested yield by increasing trichome size and density.

4. Decreasing the synthesis or accumulation of certain metabolites, such as polyphenols, ketones, terpenes and alkaloids which could affect the synthesis, accumulation, harvesting and/ or purification of the desired compounds from plant trichomes. This will be done by identifying genes in the metabolic pathway of each of the harmful compounds. DNA sequences from the genes will be used to eliminate the expression of the genes in trichome hairs of transgenic plants by antisense, co-supression or RNA inhibition methods. Alternatively it is possible to identify mutant plants or plant variants, which do not accumulate the undesired metabolites.

5. Eliminating the spreading of genetic material to the surrounding environment by for example producing sterile plants, harboring no viable pollen or seeds. This can be done by identifying sterile mutant plants (produced for example by chemical mutagenesis, physical mutagenesis or by somaclonal variation) or by silencing genes that are necessary for plant fertility.

MAKING AND USING THE INVENTION

The present invention can be utilized in the following applications:

*1. **Enhancing the expression of genes in trichome cells, by identifying novel DNA promoters and other regulatory elements.***

*2. **a. Cloning the promoter region of genes already known to be expressed in trichome cells***

Few genes were described as being expressed in trichome cells. The promoter activity of the upstream genomic region of those genes will be analyzed. Those promoters will be amplified using inverse-PCR or PCR (in case genomic sequence is already published) methods (described below) and clone to a binary vector [for example pBI101(Clontech)], upstream to the GUS reporter gene. Analysing GUS activity will determine the capability of the cloned sequences to direct gene expression to trichome cells. Below are such sequences identified as trichome potential promoters and are predicted to direct gene expression to trichome cells.

b. Identifying genes highly express in trichome cells and cloning their promoter region

Identifying genes expressing in trichomes was done here by analyzing Expressed-Sequence-Tags (ESTs) produced from mRNA extracted from trichome cells of a certain plant (tomato in this example). First clustering and assembly program (LEADS™ in this example) is done with all the available ESTs and cDNAs sequences of a certain organism (tomato in this example) from a database (NCBI genebank in this example). The program produces a database that contains a list of cluster sequences (which are a set of sequences of expressed genes such as ESTs and cDNAs, that share identical sequences thus presumably were originated from transcribing the same gene), and tissue expression data on each sequence, coming from the ascription of each EST to a cDNA library originated from a certain organ (trichome, root or fruit for example). Indeed most of the clone libraries that are submitted to NCBI include also description on the origin of the library itself, which contains expression information (i.e. tissue source). A more detailed data on the expression profile is usually exists for cDNAs which are submitted to NCBI genebank. Overall using this method, for each cluster, a limited tissue expression profile can be revealed. Database is produced that contains a subgroup of clusters

from the clusters database. For all the clusters included in the subgroup at least one sequence was originated from trichome cells (for example an EST which was collected from trichome cDNA library, namely trichome EST). The clusters in the database are ordered based on the number and percentages of sequences coming from trichome cells out of all sequences belong to the cluster. From the database we now can select trichome specific clusters (for example all 10 ESTs building the cluster are trichome ESTs), or clusters strongly express in trichomes (for example 100 ESTs out of the 300 ESTs building the cluster are trichome ESTs). The regulation of the selected genes is controlled by promoters and other regulatory elements that direct gene expression to trichomes. Those promoters will be cloned using inverse-PCR method (described below). Table 1 presents a list of clusters (genes) identified as strong, trichome specific genes. The promoters of the genes described in table 1 will further be cloned.

Table1. Trichome enhanced clusters (genes)

Cluster name	Total EST No	Trichome EST No	No of cDNAs
Trichome1	99	96	6
Trichome2	31	26	0
Trichome3	48	37	0
Trichome4	28	21	0
Trichome5	27	20	0

*3. **Directing the translated polypeptides into leucoplasts of trichome cells, using signal peptides.***

a. Proof of concept (-Can proteins be accumulated to higher concentrations in leucoplasts)

The total accumulation of proteins in leucoplasts of trichome cells will be compared to the accumulation in the cytoplasm, using signal peptides suitable for directing polypeptides into leucoplasts. Known signal peptides will be cloned upstream and in-frame to the GUS gene. Following the translation of the GUS recombinant protein,

the protein is exported to leucoplasts, where the signal peptide is cut and the mature polypeptide is accumulated. Because the protein is not accumulated in the cytoplasm, it is expected to be stored in relatively high concentrations without going through degradation processes.

b. ***Identifying and isolation of novel signal-peptides enable the targeting of proteins into leucoplasts***

Trichome specific gene sequences were analyzed to identify possible chloroplasts directing signal peptides . using ChloroP software, version 1.1 (http://www.cbs.dtu.dk/services/ChloroP/). Predicted signal peptides were amplified by RT-PCR produced from tomato total leaf RNA. The amplified sequence should be cloned upstream and in-frame to the GUS reporter gene and downstream to trichome promoters identified above. GUS enzyme accumulation should be evaluated by measuring GUS activity (15).

4. ***Increasing trichomes number and volume***

a. Trichome density can be enlarged by, for example: over-expressing genes that regulate trichome initiation, by using specific genetic background (1,16) or by optimizing day-length (16) and other environmental chemical and hormonal treatments.

b. Trichome size can be increased by over-expressing genes modifying: trichome cell size, trichome cell division rate and trichome cell ploidy number.
c. Trichome size can be increased using other non-transgenic methods such as chemical or physical mutagenesis, somaclonal variation and the induction of polyploidy.

5. ***Decreasing the synthesis or accumulation of harmful metabolites.***
a. Gene silencing

Gene prediction methods enable us to identify genes early in the metabolic pathway of the undesired compounds normally accumulated in trichome cells and on the surface of leaves. Gene prediction includes the identification in public databases, such as GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html), of orthologous

sequences originated from our plant of interest, that share high homology to known genes in the pathway, using BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/). Alternatively, other relevant information for gene prediction can include expression profile (For example- genes expressed in trichomes, i.e. posses many "trichome ESTs), protein homology and motif search, gene ontology (Intra-cellular localization prediction) and more. Candidate genes can be used for gene silencing by expressing the sequences under the regulation of strong, trichome specific promoters (see above), in either sense (direct sequence), antisense (reverse-complementary sequence) or RNAi (both direct and reverse-complement sequences adjusted by a short adaptor sequence).

b. ***Genetic background***

In the target plant of interest, it is possible to find mutant plants or different cultivars with lower levels of the harmful compounds (1,16). Those plants can be used for over-expressing the desired gene and for improving harvesting of the protein of interest. Alternatively, the plants can be crossed with the transgenic plants over-expressing the desired genes. In the next generations one can identify individual plants, which both express the gene of interest and produce low levels of harmful compounds.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Isolating and cloning of promoter sequences in a binary vector for expressing the heterologous gene in plant tissues:

All the clusters found to be highly expressed in trichomes were analyzed for ORFs using Gene Runner software version 3.05 (Hasting Software, Inc: http://www.generunner.com/). ORFs of each gene were compared to Genbank database, using Blast (http://www.ncbi.nlm.nih.gov/BLAST/) and for the highest homologous ORF the position of the ATG starting and stop codon was compared. All the sequences described herein were shown to probable have full length ORF and to include the predicted ATG starting codon.

For cloning the promoter sequences upstream to the ATG starting codon total genomic DNA is extracted from tomato leaf tissues of 4 weeks old plants, using Dneasy plant mini kit (Qiagen, Germany) following the protocol provided by the manufacturer. Inverse PCR (including gDNA digestion self ligation and PCR) is performed on genomic DNA, following common protocol (http://www.pmci.unimelb.edu.au/core_facilities/manual/mb390.asp) with slight modifications. Inverted primers were computationally selected using PRIMER3© software (EMBL) with modifications. Additional extension of 5' prime upstream sequences can be amplified using additional rounds of inverse PCR if the isolated upstream sequence is shorter than 1 kb. PCR products, purified using PCR Purification Kit (Qiagen, Germany), and DNA sequences are determined using ABI 377 sequencer (Amersham Biosciences Inc). A new set of primers is selected for the amplification of the desired promoter sequence. A 10 bp extension is added to the 5' prime end of each of the primers that includes one of the following restriction sites absent from the promoter sequence: HindIII, SalI, XbaI, BamHI or SmaI. Restriction sites are selected so the resulted product can be digested and introduced into the binary vector in the right orientation, upstream to the GUS gene. Alternatively, it is possible to replace the GUS gene for any gene of interest, such as the human Interferon-beta gene (Accession number NM_002176), by simply digestion out the GUS gene from the binary vector pBI101, using SmaI and EcI136II restriction enzymes (MBI Fermentas Inc) and ligating a blunt ended RTPCR product of the full length cDNA into the binary vector (by using methods described herein). PCR amplification is done on the newly designed primers on either genomic DNA or on the inverse-PCR product. PCR product is purified, using PCR Purification Kit (Qiagen, Germany), and digested with the restriction sites exist in the primers (Roche, using the protocol provided by the manufacturer). The digested PCR product is purified again and cloned into the binary vector pBI101 (Clontech) also digested with the same restriction enzymes as the PCR product insert, purified using PCR Purification Kit (Qiagen, Germany), treated with alkaline-phophatase (Roche) according to the manufacturer's instructions and re-purified using PCR Purification Kit (Qiagen, Germany). PCR product and open plasmid vector are ligated using T4 DNA ligase enzyme (Roche), following the protocol provided by the manufacturer.

The following sequences resemble putative promoters, originated from trichome expressed genes and predicted to direct gene expression to trichome cells:

TR8P-Promoter sequence

gctagcctaggctcagagaagcttgtcgacgaattcagattaaaatttggatctagaaggtgaggaacttttttttaacaatatat
aagtaagcattggttataatttcacaacaacattacggtaaaacctctataaattaatactcgataaattaataatccctccttaaa
ttaattatttttcctaaggaatttccgatnagggcaatgaaaaaaatcaccattttcaataaaataatgagataatatattttcaga
agacccctatataaatacatgggtcctattaatatcataaattgattattattcaaaagcataaatatatctaagataatttagtaaa
aaaatgattctattctgtttttttttttggttaaaatttaaattgtagttgaagttcatttctaacatttcatattgcttccaagagctccaa
tttgtgtcttttcgaacttcaccatagaagagttccagatgcgataagtgtttccttacgcgtaattggttccaaagttatcagtat
catattcaatcttcatcatcgacattgctttttccgatggttatccattaaattcttctaagcttatttgaaatgggagtcaatattttat
ttggccccaacacttttttcaggccacatgtatagccctttgaatcttctccaaagcttgcaat

TR9P-Promoter sequence

aagctttaca tggaaaaaca catggacaga ctaaaacact ttttttttt taataatatt gtttgcaagt gtacaccgaa
agatctacgt taatataaca taatattacg ggtaaagcta gaagtctaat tacgaatttc atgagattta ataacttta
tttttattat atttatattt aaaaattatt aaatatatac aaatttaaac tcttaaaatc attgttacaa aatttagaat
ccaaaatgtt aatattatgg tttcgcctct gctaaacatt actaatcaaa attatctttt tgtttagagc attactactg
tacaaatcta ccaagtataa atataaaagc tgttaaagaa tttccccaca cttattattc ttaatcttcc acctacccaa
tcacaaatat attaaatgag cctctaaatt tgccctattg ccgggtaata tgatctacct atcaattatt tgtaatctag
tcaaaaagat gccaaaaaaa tataatactc catctagatt gaaaattttt gtcatagaaa agaagagaaa catgataact
ttataaaata ttttacctct ggtatagttt ggatatagcg tataataata atatattaat attaataaat gatgagatta
gttatcttta gaatgcattc tatcttatgt ctggtttgat gtattaatga caattttgtt tctacaacca tgcattatta
ctgatcaatg tattgttaaa tgctaatacg ttgatttgtt atgtattagt tacatatacc tatatgtttt gtaataagaa
aaatgatgta taactaatta ataagtagta ttatcatgag taaagttatt tttctggtca gtagagagct tctaagaaca
aaaactaaat aattgtattg tatggctgct attcaaaatt ccccacctaa cgcgtcctgg aataattgat atgacttgaa
gccgcctcta aaattaaata ataattggtg cttataatgt tttacatatt atataaagca aggtatagcc caatgaattt
tcattcaaaa gctagcaaca

Isolating and cloning in a binary vector of DNA sequences corresponding to chloroplasts/ leucoplasts signal peptides, upstream and in-frame to the heterologous gene:

All the above sequences were analyzed for ORFs using Gene Runner software version 3.05 (Hasting Software, Inc: http://www.generunner.com/). ORFs of each gene were compared to Genbank database, using Blast (http://www.ncbi.nlm.nih.gov/BLAST/) and for the highest homologous ORF the protein length and positions of the starting and terminating codons were compared. All the sequences described herein were predicted to have full length ORFs and to include the apparent ATG starting codon. cDNA sequences harboring signal peptides on their N-terminal translated protein were identified using ChloroP software, version 1.1 (http://www.cbs.dtu.dk/services/ChloroP/).

For cloning the signal peptide sequences extraction of total RNA from tomato root and leaf tissues of 4 weeks old plant is performed, using Tri Reagent (Molecular Research Center, Inc) following the protocol provided by the manufacturer (http://www.mrcgene.com/tri.htm). For producing cDNA molecules from tissue mRNA, a reverse-transcriptase (RT) reaction is performed using: M-MuLV RT enzyme (Roche), $T_{16}NN$ DNA primer, following protocol provided by the manufacturer. cDNA is amplified by PCR with PFU proof reading DNA polymerase enzyme (Promega-http://www.promega.com/pnotes/68/7381_07/7381_07.html) using the protocol provided by the manufacturer. The resulting PCR blunt ended products, purified using PCR Purification Kit (Qiagen, Germany), is cloned into the SmaI blunt restriction site of the binary vector pBI101 (Clontech) as described above. To avoid mutations produced in the PCR reaction and to identify the orientation of the introduced signal peptide, sequencing for the insert is done. Primers used for sequencing the inserts of a pBI101 binary vector can be: 5'- CTGGTGATTTTGCTGGCTCTA -3' (forward) and 5'-AAGTTGGGTAACGCCAGGGT-3' (reverse).

The following sequences resemble putative signal peptides, originated from trichome expressed genes and direct polypeptide to chloroplasts/ leucoplasts, as was predicted using ChloroP software:

TR3 (partial)

GACCCCTCCACTCCCAAAAACAACACACAATATTCAAGGATGATAGTTGG
CTATAGAAGCACAATCATTACCCTTTCTCATCCTAAGCTAGGCAATGGGA
AAACAATTTCA

TR4

ATGAGTTCTTTGGTTCTTCAATGTTGGAAATTATCATCTCCATCTCTGATTT
TACAACAAAATACATCAATATCCATGGGTGCATTCAAAGGTATTCATAAA
CTTCAAATCCCAAATTCACCTCTGACAGTGTCTGCT

Agrobacterium transformation of binary plasmids harboring the genes of interest:

Agrobacterium tumefaciens strains GV301, GV303 or LB4404 competent cells are transformed using 1–2 µl of ligation reaction mixture by electroporation, using a MicroPulser electroporator (Biorad), 0.2 cm cuvettes (Biorad) and EC-2 electroporation program (Biorad). *Agrobacterium* cells are grown on LB at 28°C for 3 hrs and plated on LB-agar plates supplemented with the antibiotics gentamycin 50 mg/L (for Agrobacterium strains GV301, GV303), streptomycin 250 mg/L (for Agrobacterium strain LB4404), and kanamycin 50 mg/L (Sigma). Plates are then incubated at 28°C for 48 hrs. *Agrobacterium* colonies can be subsequently used for *Arabidopsis* or tomato plant transformation.

Plant transformation and cultivation:

Arabidopsis thaliana Columbia (T_0 plants) are transformed using the Floral Dip procedure described by Clough SJ and Bent AF (17) and by Desfeux C *et al.* (18), with minor modifications. Briefly, T_0 Plants are sown in 250 ml pots filled with wet peat-based growth mix. The pots are covered with aluminum foil and a plastic dome, kept at 4°C for 3–4 days, then uncovered and incubated in a growth chamber at 18–24°C under 16/8 hr light/dark cycles. The T_0 plants are ready for transformation six days before anthesis.

Single colonies of *Agrobacterium* (Strain GV301 or GV303) carrying plant DREs are cultured in LB medium supplemented with kanamycin (50 mg/L) and gentamycin (50 mg/L). The cultures are incubated at 28^{0}C for 48 hrs under vigorous shaking and centrifuged at 4000 rpm for 5 minutes. The pellets comprising *Agrobacterium* cells are resuspended in a transformation medium which contained half-strength (2.15 g/L) Murashig-Skoog (Duchefa); 0.044 µM benzylamino purine (Sigma); 112 µg/L B5 Gambourg vitamins (Sigma); 5% sucrose; and 0.2 ml/L Silwet L-77 (OSI Specialists, CT) in double-distilled water , at pH of 5.7.

Transformation of T_0 plants is effected by inverting each plant into an *Agrobacterium* suspension such that the above ground plant tissue is submerged for 3-5 seconds. Each inoculated T_0 plant is immediately placed in a plastic tray, then covered with clear plastic dome to maintain humidity and kept in the dark at room temperature for 18 hrs to facilitate infection and transformation. Transformed (transgenic) plants are then uncovered and transferred to a greenhouse for recovery and maturation. The transgenic T_0 plants are grown in the greenhouse for 3-5 weeks until siliques were brown and dry, then seeds are harvested from plants and kept at room temperature until sowing

For generating T_1 and T_2 transgenic plants harboring the genes seeds collected from transgenic T_0 plants are surface-sterilized by soaking in 70% ethanol for 1 minute, followed by soaking in 5% sodium hypochloride and 0.05% triton for 5 minutes. The surface-sterilized seeds are thoroughly washed in sterile distilled water then placed on culture plates containing half-strength Murashig-Skoog (Duchefa); 2% sucrose; 0.8% plant agar; 50 mM kanamycin; and 200 mM carbenicylin (Duchefa). The culture plates are incubated at 4^0C for 48 hours then transferred to a growth room at 25^0C for an additional week of incubation. Vital T_1 *Arabidopsis* plants are transferred to a fresh culture plates for another week of incubation. Following incubation the T_1 plants were gently collected from culture plates and planted in growth mix contained in 250 ml pots. The transgenic plants are allowed to grow in a greenhouse to maturity. Seeds harvested from T_1 plants are cultured and grown to maturity as T_2 plants under the same conditions as used for culturing and growing the T_1 plants.

Tomato transformation is done following Curtis *et al.* (19), with slight modifications

GUS staining

Gus staining of T1 and T2 arabidopsis and tomato plants is done following routine protocol (15).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad

scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

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WHAT IS CLAIMED IS:

1. Constructs, construct systems and methods for expressing exogenous polynucleotides in trichomes essentially as described herein.

2. A plant having trichomes comprising an exogenous polypeptide.

3. The plant of claim 2, wherein said exogenous polypeptide is located within a subcellular compartment of said trichomes

4. The plant of claim 3, wherein said subcellular compartment is a leucoplast.

5. Trichome specific promoters TR8P and TR9P as described herein and homologues and mutants thereof.